Remarks

Claims 10-16, 22, and 23 have been canceled, claims 1, 4, 5, 7, 9, 17, and 21 have been amended, and new claim 24 has been added. No new matter has been added by way of these amendments. Claims 1-9, 17-21, and 24 are pending in the above-captioned application.

Applicants submit herewith certified copies of 35 U.S.C. § 119 priority applications, German application P 43 17 459.0, and German application P 43 29 756.0.

In response to the restriction requirement, Applicants affirm the provisional election of the invention of Group I, claims 1-9 and 17-21, which are directed to methods of expressing IFN-α in *E. coli*, obtaining purified protein, and recombinant vectors for the practice of the claimed methods. Claims 10-16, drawn to methods of purifying IFN-α, have been canceled without prejudice to the filing of a divisional application.

In response to the Examiner's request, Applicants have submitted herewith a copy of the Information Disclosure Statement, form PTO-1449, and copies of cited non-patent documents originally filed on November 15, 1994 (Paper No. 7).

Applicants wish to thank the Examiner for the corrections which were made to the Sequence Listing for the above-captioned application.

Support for the amendment to the specification at p. 4, wherein Applicants have clarified that the exchange chromatography steps referred to at ll. 20-21 are first cation and then anion exchange chromatography, may be found at p. 4, ll. 7-8. Thus, no new matter is added by way of this amendment.

Support for the amendment to the specification at pp. 8-9, wherein the term "nucleotide" is replaced with "polynucleotide," may be found throughout the application as filed. Thus, no new matter is added by way of these amendments.

In the Office Action mailed September 8, 1995, the Examiner made two objections to informalities in the specification, two objections to the claims, and six rejections to the claims. In response thereto, Applicants respectfully submit the following remarks.

I. The Amendment to the Specification has Overcome the Objections to the Specification

A. The Examiner has objected to the specification because of the confusion created by the labeling at pages 7-8 of apparently identical amino acid sequences as both SEQ ID NO: 5 and SEQ ID NO: 7. Applicants have amended the application to delete reference to the sequence designated SEQ ID NO: 7 in the application as filed and to correct references to SEQ ID NOs: 8-12 to refer instead to SEQ ID NOs: 7-11. The sequence listing has been amended to eliminate the sequence designated SEQ ID NO:7 in the application as filed, and to change the numbering of subsequent SEQ ID NOs: 8-12 to SEQ ID NOs: 7-11. Applicants have cancelled the sequence listing, submitted an amended computer readable copy of the sequence listing, and have amended the specification to direct the entry of the paper copy of the amended sequence listing between the specification and claims of the above identified application. Applicants would like to point out to the Examiner that the sequence specified in new claim 24 as SEQ ID NO: 7 corresponds to SEQ ID NO: 8 in the application as filed.

In accordance with 37 C.F.R. § 1.821(f), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith are the same.

In accordance with 37 C.F.R. § 1.821(g), applicants respectfully submit that no new matter has been added by way of the amendment to the sequence listing.

In light of these amendments, Applicants respectfully request that this objection to the specification be withdrawn.

B. The Examiner has noted the use of trademarks in the instant application, and has requested that the application be reviewed in order to properly identify all trademarks. Applicants have amended the specification to identify each trademark referred to in the application. Specifically, all references to Sepharose™, DEAE Sepharose™, and DEAE Sepharose Fast Flow™ have been denoted as trademarks and capitalized. Applicants would like to point out to the Examiner that generic terminology is not available for the Sepharose™ products of Pharmacia, as these products are beaded agarose matrices whose properties cannot fully be described by their chemical composition, as could a chemical reagent. Further, the term "Sepharose™" has a fixed and definite meaning to those of ordinary skill in the art, and so it is permissible under MPEP § 608.01(v) to refer to it solely by its name. Applicants have also amended the specification to appropriately identify other trademarks, including "Bluescribe™," "Toyopearl™," "Fast Flow™," "Supersphere™," and "Tween™ 20." Thus, Applicants submit that the objection to the specification has been overcome and hence request that it be withdrawn.

II. The Objections to the Claims have been Overcome by Amendment

A. As suggested by the Examiner on page 4 of the Office Action, claim 1 has been amended to recite that "a nucleotide sequence encoding the signal peptide" of the STII gene product is a component of the expression vector. Support for this amendment may be found on p. 6, ll. 12-13 and ll. 18-20 of the specification (discussing construction of a vector for expression of IFN-α, and stating that a nucleotide sequence which codes for the signal peptide of the STII gene is known). In light of this amendment, Applicants respectfully request that the objection to claim 1 be withdrawn.

B. The Examiner has objected to claims 1 and 23 as being identical in scope, and to claims 21 and 22 as being *verbatim* identical.

Applicants have canceled claims 22 and 23, and have added new claim 24, in which the specified sequence includes 285 bases upstream of the DNA encoding the STII leader peptide. Support for this amendment may be found on p. 9, l. 22, p.10, l. 7, and at Fig. 2B. In light of these amendments, Applicants respectfully request that the objection to the claims be withdrawn.

III. The Amendment to the Claims has Overcome the Rejection Under 35 U.S.C. § 112, Second Paragraph

The Examiner has rejected claims 1-9, 21 and 22 as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Applicants submit that the present amendment to the claims has overcome this basis for rejection for the reasons summarized below.

A. The Examiner has rejected Claim 1 and the claims dependent therefrom under § 112, second paragraph, stating that claim 1 is indefinite, as it recites that the "signal sequence of the [STII] gene" is a component of the vector employed, although a signal sequence is a translation product and hence cannot be a feature of the vector.

Applicants have amended claim 1 to recite that the vector comprises "a nucleotide sequence encoding the signal peptide for...STII." Support for this amendment may be found at p. 6, ll. 12-14. Applicants therefore submit that the basis for rejection of claim 1 under § 112, second paragraph, has been overcome, and respectfully request that the Examiner withdraw the rejection thereto.

B. The Examiner has rejected claim 4 as being incomplete and indefinite for reciting in both steps (c) and (d) that "exchange chromatography" is performed, and has rejected claims 6 and 7 as lacking antecedent basis for referring, respectively, to "said cation exchange chromatography" and "said anion exchange chromatography."

Applicants have amended claim 4 to recite that in step (c), cation exchange chromatography is carried out and that in step (d), anion exchange chromatography is carried out. Support for this amendment may be found at p. 4, ll. 7-8. Thus, claim 4 is no longer incomplete and indefinite, and claims 6 and 7 no longer lack antecedent basis. Thus, Applicants respectfully request that the rejection of claims 4, 6 and 7 under 35 U.S.C.§ 112 be withdrawn.

C. The Examiner has rejected claims 9, 21 and 22 as being vague and indefinite for using the phrase "consisting essentially of" to refer to features of single molecules.

Applicants have amended claims 9 and 21 to replace the phrase "consisting essentially of" with "comprising" as suggested by the Examiner, and have canceled claim 22. Thus, Applicants respectfully submit that this basis for rejection must be withdrawn.

D. The Examiner has also rejected claims 21 and 22 under § 112 for claiming sequences which are "more than about 70% homologous" to the referenced sequence, stating that homology is employed in the art to reflect evolutionary relationships, and is meaningless when recited in a quantitative context. Applicants respectfully traverse this basis for rejection of claim 21, and submit that the cancellation of claim 22 renders its rejection moot.

The term homology is routinely used by those of skill in the art to designate in a quantitative manner the degree of similarity that exists between two fragments of DNA. As stated in the standard genetics text *Genes and Genomes* (Singer *et al.*, University Science Books, pp. 625-626, 1991, copy attached):

> ...the term ***homologous*** is often also used to describe similar but not identical DNA segments. Frequently, a qualitative modifier is applied to indicate the extent of identity (e.g., highly homologous meaning highly similar). *When the actual sequences are known, quantitative statements can be made. Thus, two DNA sequences are described as being 70 percent homologous (or 70 percent similar) if 70 percent of their bases are identical and colinear* . . . Although DNA sequences that are 70 or even 30 percent similar are likely to be derived from a common ancestral DNA sequence, they may not be so related. Thus, the common use of percent homology in the molecular genetics literature. . .is somewhat ambiguous. The term percent similarity. . .avoids the ambiguity *but is only rarely found in the literature.* (emphasis added)

Thus the use of the term "homologous" in claim 21 does not render the claim indefinite, as the terminology is quite familiar to the audience to whom the present patent application is

directed, and is in fact preferred by molecular geneticists over the designation "percent similarity."

However, in order to eliminate any possible ambiguity caused by the reference to "said homologous," and to more clearly define the subject matter which Applicants regard as their invention, Applicants have amended claim 21 so that it recites that the claimed vector comprises either a sequence (specified in the claim) encoding interferon-α, or a sequence "encoding interferon-α which has more than about 70% sequence identity with this sequence." Applicants submit that in light of the amendments and the remarks *supra*, the rejection of claim 21 under 35 U.S.C. § 112 must be withdrawn.

IV. The Claims are not Obvious Over the Prior Art

A. The Examiner has rejected claims 1, 2, 17-18 and 23 under 35 U.S.C. § 103 as being unpatentable over Miyake *et al.* (*JB* 97:1429-36, 1985) in view of Fuh *et al.* (*JBC* 265: 3111-15, 1990) and Morioka-Fujimoto *et al.* (*JBC* 266: 1728-32, 1991). Applicants respectfully traverse this basis for rejection as to claims 1, 2, 17 and 18, and submit that the cancellation of claim 23 renders its rejection moot.

The Examiner states that it would be obvious to construct an expression vector for IFN-α according to Miyake, replacing the phoA signal peptide-encoding sequence with the STII signal peptide-encoding sequence to make the expression cassette taught by Fuh, because Fuh teaches that this cassette affords high levels of expression of a heterologous protein, and because Morioka-Fujimoto teaches that the STII signal sequence is the most

effective of those tested for production of a heterologous protein. Applicants respectfully disagree.

1. The Prior Art does not Suggest the Claimed Invention

Applicants respectfully submit that the cited references, alone or in combination, do not suggest the claimed invention. Applicants assert that the Examiner's statement that it would be obvious to substitute the STII sequence of Fuh and Morioka-Fujimoto for the signal peptide-encoding sequence used in the IFN-α expression vector of Miyake to yield the claimed invention can only be the result of hindsight reconstruction. One of ordinary skill in the art, taking into account the entire body of prior art, would find nothing to suggest that the solution to the problem of expression/secretion of IFN-α from a bacterial host would be to construct the expression vector used so successfully by Applicants.

Applicants respectfully disagree that the successful expression and secretion of a mammalian protein from a bacterial host can be expected merely by fusing the gene of interest to a bacterial signal peptide-encoding sequence which has been successful for expression/secretion of a different mammalian protein. In order to secrete a polypeptide, the polypeptide must be recognized by the cellular secretion machinery and processed by the signal peptidase. The efficiency of secretion is thus greatly dependent upon how well the signal peptidase can interact with the protein, which in turn depends upon the characteristics and structural constraints of the protein to be secreted. Applicants stress that the expression products of Miyake, Fuh, Morioka-Fujimoto and the present invention are each artificially

constructed fusion proteins comprising a bacterial signal sequence and a mammalian protein. Thus, the properties of each of these polypeptides, along with their attendant structural constraints, are unique, and their tertiary structures have not been established, as they are artificial constructs never created before these expression experiments. Human growth hormone (hGH), the protein produced and secreted in Fuh, is a 22 kD protein composed of 191 amino acids with an isoelectric point of 4.9 (J. E.F. Reynolds, ed., *Martindale*, The Pharmaceutical Press, London, p. 1270 (28th ed., 1982), copy attached). In contrast, interferon-α has a molecular weight of 19 kD, and is composed of 165-166 amino acids having an isoelectric point of 5.5-6.5 (Pestka, S., *et al.*, *Ann. Rev. Biochem. 56*: 730, 1987, copy attached). Hence, Applicants respectfully assert that the Examiner's belief that successful secretion of one "heterologous" protein via the STII leader peptide gives rise to a high probability that the "heterologous" protein IFN-α will likewise be successfully secreted is incorrect. Applicants assert that one of ordinary skill in the art would not assume that the fact that a specific prokaryotic signal sequence, STII, promotes efficient secretion of a specific mammalian protein, hGH, would mean that IFN-α would also be likely to be expressed and secreted efficiently if fused to the STII signal peptide, as nothing in Fuh or Morioka-Fujimoto predicts that the same signal sequence would efficiently promote secretion of a protein having the special characteristics of IFN-α.

Not any combination of a mammalian gene with a bacterial signal sequence leads to an efficient secretion system. Applicants wish to emphasize that others have unsuccessfully attempted to construct an effective IFN-α secretion vector by utilizing bacterial signal

sequences. As outlined in the specification of the present application at p. 2, ll. 7-20, Breitling *et al.* (*Mol. Gen. Genet. 217*:384-391, 1989) were unsuccessful in their attempt to achieve secretion of IFN-α via the bacterial signal peptide sak42D. The attempt of Miyake *et al.* was also an unsuccessful attempt to produce IFN-α in a quantity sufficient for production purposes. These failed attempts would indicate to one of ordinary skill that simply fusing the STII bacterial signal peptide to IFN-α would be unlikely to be any more successful than had been the previous attempts. Clearly, the solution to the problem solved by the present invention was not obvious to those of ordinary skill in the art at the time the invention was made.

Further, Applicants assert that the experimental findings of Morioka-Fujimoto do nothing to indicate that the problems encountered by Breitling *et al.* and Miyake *et al.* could be cured by the fusion of the STII signal peptide to IFN-α. Morioka-Fujimoto *et al.* established that the STII signal sequence was the best of two wild-type bacterial signal sequences, LTA and STII, tested for the secretion of rhEGF from *E. coli.* These experiments did not compare the STII sequence to other bacterial signal sequences such as *pho*A or *sak*42D which have been used in recombinant expression systems. Hence one would not conclude that, for the purposes of expression and secretion of IFN-α, the STII sequence would give better results than those obtained by Breitling *et al.* or Miyake *et al.* The Morioka-Fujimoto reference merely teaches that the STII signal sequence is more efficient than the LTA signal sequence for the secretion of one specific polypeptide, rhEGF.

Applicants also disagree with the Examiner's position that the Morioka-Fujimoto *et al.* reference would have been deemed especially pertinent to the problem that Applicants were facing because that reference dealt with the production of a cytokine. The designation "cytokine" is merely a generic term for soluble molecules which mediate interactions between cells, and does not imply a common structure. Although some cytokines are part of related "families," the majority are genetically unrelated, dissimilar molecules. This is the case for epidermal growth factor, the protein expressed and secreted in the Morioka-Fujimoto reference, and IFN-α. EGF is a 53 amino acid polypeptide involved in mitogenesis, while IFN-α molecules comprise 165-166 amino acids and interfere with viral replication. Hence, one of ordinary skill in the art would not expect a bacterial expression system for rhEGF to be any more successful for expression/secretion of IFN-α than any other bacterial expression/secretion system. Hence, although combining the *pho*A promoter of Miyake *et al.* with the STII signal peptide-encoding sequence of Fuh *et al.* and Morioka-Fujimoto *et al.* would be one possible bacterial expression/secretion system to be tried for production of IFN-α, it would not be obvious that this choice would be more successful for IFN-α production than any of the other possible bacterial promoter/signal peptide-encoding sequence combinations which could be tried.

Thus, Applicants assert that at best, it would have been "obvious to try" the substitution of the STII signal sequence of Fuh for the phoA signal peptide-encoding sequence in the IFN-α expression vector of Miyake *et al.* As the Examiner is no doubt aware, "obvious to try" is *not* the standard by which unpatentability under 35 U.S.C. § 103 is

determined. As was stated in *In re O'Farrell*, where it is ". . .'obvious to try'. . . to vary all parameters or try each of numerous possible choices until one possibly [arrives] at a successful result, where the prior art gave . . . no direction as to which choice was likely to be successful," obviousness under § 103 has not been established.

Applicants respectfully assert that the Examiner has not established that the claimed invention is *prima facie* obvious over the cited combination of references, and respectfully request that the rejection under 35 U.S.C. § 103 over Miyake *et al.* in view of Fuh *et al.* and Morioka-Fujimoto *et al.* be withdrawn.

B. The Examiner has rejected claims 8, 9, and 20-22 under 35 U.S.C. § 103 as being unpatentable over Miyake, Fuh, and Morioka-Fujimoto as applied to claims 1, 2, 17, 18, and 23 above, and further in view of Hauptmann *et al.* (US 4,917,887). Applicants respectfully traverse this basis for rejection as to claims 8, 9 and 20-21, and submit that the cancellation of claim 22 renders its rejection moot.

The Examiner states that it would have been obvious to one of ordinary skill in the art to replace the hIFN-α sequence of Miyake with the hIFN-α2 amino acid and corresponding nucleic acid sequences disclosed by Hauptmann, which are identical to SEQ ID NOs:5 and 6, respectively, of the present application, in a vector incorporating the STII signal peptide sequence, as suggested by Fuh and Morioka-Fujimoto, because Hauptmann evidences that the disclosed IFN-α2 species is useful. Applicants respectfully disagree.

For the reasons summarized above, Applicants assert that the use of an expression vector comprising the *pho*A promoter, the STII signal peptide-encoding sequence, and the

IFN-α gene would not have been obvious to one of ordinary skill in the art at the time the invention was made. Thus, substitution of the IFN-α2 gene for that of IFN-α into such an expression vector would also not be obvious. Thus, Applicants assert that the rejection of claims 8, 9, and 20-21 under 35 U.S.C. § 103 over the cited references has been overcome, and respectfully request that the rejection be withdrawn.

C. The Examiner has rejected claims 3 and 19 under 35 U.S.C. § 103 as being unpatentable over Miyake, Fuh, and Morioka-Fujimoto as applied to claims 1, 2, 17, 18 and 23, above, and further in view of Stephens *et al.* (US 4,769,327). Applicants respectfully traverse this basis for rejection.

The Examiner states that it would have been obvious to one of ordinary skill in the art to further incorporate into an IFN-α expression vector incorporating the STII signal peptide sequence a ribosome binding site (RBS) which is native to the host cell to be employed. Applicants respectfully disagree.

For the reasons summarized above, Applicants assert that the use of an expression vector comprising the *pho*A promoter, the STII signal peptide-encoding sequence, and the IFN-α gene would not have been obvious to one of ordinary skill in the art at the time the invention was made. Thus, despite the fact that Stephens teaches that it is desirable to include a RBS native to the host cell into an expression vector, the additional incorporation of the *E. coli* STII RBS into such an expression vector would also not be obvious, as it would not have been obvious to construct that vector even without a RBS. Thus, Applicants assert that the

rejection of claims 3 and 19 under 35 U.S.C. § 103 over the cited references has been overcome, and respectfully request that the rejection be withdrawn.

D. The Examiner has rejected claims 4-7 under 35 U.S.C. § 103 as being unpatentable over Miyake, Fuh, and Morioka-Fujimoto as applied to claims 1, 2, 17, 18 and 23, above, and further in view of Protasi *et al.* (US 5,066,786) and Higashi *et al.* (US 4,828,990). Applicants respectfully traverse this basis for rejection.

The Examiner states that it would have been obvious to one of ordinary skill in the art at the time the invention was made to purify IFN-α produced according to the suggestion of Miyake, Fuh, and Morioka-Fujimoto by any methods known in the art for the purification of IFNs, which would include adsorption on a silicious material as suggested by Protasi, or anion exchange, cation exchange, or hydrophobic interaction chromatography, as suggested by Higashi.

Applicants submit that as the expression system for IFN-α claimed in claim 1 by Applicants is not obvious under 35 U.S.C. § 103, claims 4-7 dependent thereon cannot be deemed obvious merely because they incorporate protein purification steps which may have been known individually in the art. Thus, Applicants submit that claims 4-7 are not obvious under 35 U.S.C. § 103 over the cited references, and respectfully request that the rejection be withdrawn.

Conclusion

Applicants respectfully submit that all the bases for objection to the specification and claims, as well as rejection of the claims, have been overcome by the above amendments and remarks. Reconsideration of the application is respectfully requested, and passage of the application to issuance is earnestly solicited.

Respectfully submitted,

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intergenic regions. They make up as much as 0.5 percent of the genomes. Although the full significance of the repeats is not known, they may be sites for DNA gyrase action because gyrase binds to them with high affinity. In addition, prokaryotic DNA may contain repeated units several kilobase pairs in length. Some of these are genes for rRNA; others are transposable elements (Section 10.2a). These repeats, as well as fortuitous and quite short sequence duplications, foster the spontaneous formation of deletions and tandem repetitions by unequal homologous crossing-over. Even considering the repetitious elements in prokaryotes, however, the situation in many eukaryotes is quite distinctive. Fifty percent or more of these genomes are frequently composed of reiterations, and there may in fact be very few truly unique DNA segments. It is important to distinguish between a unique DNA segment (or sequence) and a unique gene. A specific functional gene that is present only once per haploid genome and is thus unique may belong to a family of repeated sequences that includes related genes as well as pseudogenes.

Some repeated sequence families take the form of uninterrupted tandem arrays of the repeating units (Figure 9.1). In others, the family members are clustered but have the directly or inversely repeated units separated by other kinds of sequence. The members of still other families are dispersed to distant places in the genome, including different chromosomes; in some cases, tandem arrays are themselves dispersed.

Variability of repeated sequence family members The nucleotide sequences of members of a particular repeat family may be identical or may vary in one or more base pairs. In the latter case, the family is said to be **polymorphic**. In some families, the divergence is considerable. The terms **subfamilies** and **superfamilies** are used to describe sets of family members with greater and lesser similarity, respectively. The extent of divergence between different family members is estimated with increasing accuracy by four methods: (1) comparing restriction endonuclease maps, (2) measuring their ability to anneal together, (3) measuring the thermal stability of hybrid duplexes formed between family members, and (4) comparing actual nucleotide sequences. Stringent annealing conditions (usually relatively high temperature, about 65°C, and relatively low salt, about 0.45 M NaCl) generally allow duplex formation only between DNA segments whose nucleotide sequences are complementary in at least 85 percent of the nucleotide residues. Lowering the stringency by decreasing the temperature or increasing the salt concentration permits annealing of more distantly related segments, but the lower limit of detectability is about 70 percent complementarity. The stability of the heteroduplexes formed is estimated by measuring their conversion to single strands as a function of temperature (Section 1.1f). As a general rule of thumb, the T_m is lowered 1°C for every 1 percent of mismatched base pairs.

Duplex DNA segments are said to be homologous if their sequences are identical. However, the term **homologous** is often also used to describe similar but not identical DNA segments. Frequently, a qualitative modifier is applied to indicate the extent of identity (e.g., highly homologous meaning highly similar). When the actual sequences are known, quantitative statements can be made. Thus, two DNA segments are described as being 70 percent homologous (or 70 percent similar) if 70 percent of their base pairs (or nucleotide residues) are identical and colinear. This

usage derives from the concept of homologous chromosomes, but it i different from that typical of the literature on biological evolution. Ther homologous generally means that morphological structures or DNA se quences share a common ancestor, and the idea of percent homology i thus meaningless. Although DNA sequences that are 70 or even 30 per cent similar are likely to be derived from a common ancestral DNA se quence, they may not be so related. Thus, the common use of percen homology in the molecular genetics literature and in this book is some what ambiguous. The term percent similarity, which we also use, avoid the ambiguity but is only rarely found in the literature.

The size of repeated sequence families The number of related units in dif ferent eukaryotic sequence families varies from two to millions, and th **copy number** is frequently different in the genomes of different species Although the actual copy number is the most informative description many families can now only be described as highly, moderately (or middle) or infrequently repeated. Historically, these classifications were associatec with ranges of Cot values (Section 7.5b). For example, mammalian highly repetitive DNA (10^5 copies or more) was defined by Cot values less thar 0.1, middle repetitive (between 10^2 and 10^5 copies) by Cot values be tween 0.1 and 100, and infrequently repetitive (fewer than 100 copies by Cot values greater than 100.

Consensus sequences When a repeated sequence family has many mem bers, each of which differs from the others at only a few positions in the nucleotide sequence, it is often convenient to define an average, or **consensus**, sequence for all members of the family. A consensus sequence represents the most frequent base at each position in the population of family members and is not necessarily identical to the sequence of any single member. There are two ways to obtain a consensus sequence. Sometimes it is possible to isolate a large population of the repeating units free from unrelated sequences and subject the mixture to sequence analysis. Even if each molecule in the population differs from the others at a few positions, a unique nucleotide sequence is often obtained because the sequencing methods do not detect the small percentage of divergent nucleotides at any single position (Section 7.2). In the second approach, the sequences of a number of individually cloned family members are compared, and the most frequent base at each residue is calculated.

It is helpful to remember that the definition of a family of repeated sequences is pragmatic. In the absence of actual sequence data, a family is generally taken to be all sequences that anneal to one another, or to a single cloned family member, under stringent conditions. Generally, this means that sequences that diverge 15 to 20 percent from a consensus sequence are barely detectable, and other, more divergent sequences are not counted as family members. Also, the identification of repeated sequence families by reannealing systematically omits families whose members are too short to reanneal. For example, the 5'-ACT(A or T)$_n$TA family members that precede tRNA genes (Section 9.2d) are not detected. Often, nucleotide sequence data show that two DNA sequences that do not anneal with one another are sufficiently similar (e.g., 30 to 50 percent) to be grouped within a family (or superfamily).

MARTINDALE

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daily for 10 days commencing 5 days before [illegible]mination of human menopausal gonadotrophin and chorionic gonadotrophin, inhibited ovulation.— A. Jacobson and J. R. Marshall (letter), *Lancet*, 1968, *2*, 286.

Uses. Follicle-stimulating hormone followed by chorionic gonadotrophin is used in the treatment of anovulatory infertility due to insufficient gonadotrophins. Follicle-stimulating hormone is administered to induce follicular maturation and endometrial proliferation, and is followed by treatment with chorionic gonadotrophin to stimulate ovulation and corpus luteum formation. It is considered that follicle-stimulating hormone alone does not produce full follicular maturation; a preparation containing follicle-stimulating hormone and luteinising hormone (human menopausal gonadotrophin; HMG) in an equal number of units is usually used. Clomiphene (p.1406) can also be used to induce ovulation; amenorrhoeic women with hyperprolactinaemia may be treated with bromocriptine (p.894).

The dosage and schedule of treatment must be determined according to the needs of each patient; it is usual to monitor response by studying the patient's urinary oestrogen excretion. Follicle-stimulating hormone may be given daily by intramuscular injection in a dose of 75 units with 75 units of luteinising hormone, until an adequate response, judged on the basis of daily oestrogen determinations, is achieved, followed after 1 or 2 days by chorionic gonadotrophin (see p.1268). Alternatively, three doses may be given on alternate days followed by chorionic gonadotrophin on the eighth day.

Infertility. A detailed analysis was made of 78 pregnancies and births resulting from the induction of ovulation in 68 patients with long-term sterility. All women received treatment with chorionic gonadotrophin and follicle-stimulating hormone with luteinising hormone except 2 who were given clomiphene citrate and follicle-stimulating and luteinising hormones. The pregnancies resulted in 122 births, including 47 single, 23 twin, 5 triplet, 2 quadruplet, and 1 sextuplet set. Ten women developed mild or severe hyperstimulation and 3 of 4 children with major malformations were born to mothers who suffered hyperstimulation. The hyperstimulation syndrome was characterised by an abnormal hormone excretion and disturbances in the normal homoeostasis of the body. Intra-uterine growth and postnatal development of the children born after treatment were normal. The abortion-rate was 29% and the foetal and neonatal death-rate was 27%.— M. Hack *et al.*, *J. Am. med. Ass.*, 1970, *211*, 791.

Anovulatory infertility in 59 patients was treated according to a treatment-orientated diagnostic classification of their amenorrhoea. Patients with oestrogen deficiency who did not respond to clomiphene and who did not have raised serum concentrations of follicle-stimulating hormone or prolactin were treated with follicle-stimulating hormone with luteinising hormone and chorionic gonadotrophin; 11 of 12 patients subsequently conceived. Six similar patients in whom weight loss was a problem were encouraged to increase their weight and 5 conceived without the use of gonadotrophins, although 2 were given clomiphene to correct luteal deficiency when menstruation had resumed.— M. O. R. Hull *et al.*, *Br. med. J.*, 1979, *1*, 1257.

Further references: B. T. Tyler, *J. Am. med. Ass.*, 1968, *205*, 16; A. D. Tsapoulis and A. C. Crooke, *Lancet*, 1968, *2*, 1321; A. C. Crooke *et al.*, *ibid.*, 180; *Br. med. J.*, 1972, *4*, 167; Human Pituitary Advisory Committee, *Med. J. Aust.*, 1975, *2*, 549; J. D. Ellis and J. G. Williamson, *Br. J. Obstet. Gynaec.*, 1975, *82*, 52; J. Evans and L. Townsend, *Am. J. Obstet. Gynec.*, 1976, *125*, 321; D. Rabinowitz *et al.*, *New Engl. J. Med.*, 1979, *300*, 126; O. Oelsner *et al.*, *Fert. Steril.*, 1978, *30*, 538; C. M. March *et al.*, *Obstet. Gynec.*, 1979, *53*, 8.

Male infertility. In 11 infertile men with severe oligospermia, treatment with follicle-stimulating and luteinising hormones increased the sperm count in 5 patients and definitely improved the motility of spermatozoa in 7. The wives of 2 patients in the latter group subsequently became pregnant.— J. M. Danczis and L. Barrinos, *Fert. Steril.*, 1967, *18*, 788. per *J. Am. med. Ass.*, 1969, *207* (Jan. 15), A212.

Ovarian-function test. As a test of ovarian function prior to induction of ovulation, patients were given by injection 3 ampoules of follicle-stimulating hormone 75 units with luteinising hormone 75 units daily for 3 days. Where ovarian function could be stimulated, a rise in urinary oestrone occurred and reached a peak 3 to 4 days after the treatment. The urinary oestrone concentration 2 days after treatment was compared with the concentration prior to treatment; increases of 6 to 40 μg per 24 hours were considered satisfactory and an increase of 41 μg or more per 24 hours indicative of ovarian over-responsiveness. Of 11 patients adjudged satisfactory by the test, 10 subsequently ovulated when treated with either clomiphene or gonadotrophin.— R. I. Cox *et al.*, *Lancet*, 1966, *2*, 888.

Preparations

Menotrophin Injection *(B.P.).* A sterile solution of menotrophin in Water for Injections prepared by dissolving, immediately before use, the contents of a sealed container (Menotrophin for Injection) in the requisite amount of Water for Injections. pH 6 to 8. The sealed container may contain added inert substances.

Menotropins for Injection *(U.S.P.).* A sterile freeze-dried mixture of menotropins *U.S.P.* and suitable excipients; it may contain an antimicrobial agent. pH of the reconstituted solution 6 to 7.

Pergonal *(Serono, UK).* Ampoules each containing, as powder for preparing solutions, follicle-stimulating hormone 75 units and luteinising hormone 75 units with lactose 10 mg, supplied with 1-ml ampoules of sodium chloride injection. (Also available as Pergonal in *Austral., Ger., Neth., S.Afr., USA*).

Other Proprietary Names

Humegon *(Austral., Belg., Denm., Fr., Ger., Neth., Norw., S.Afr., Swed., Switz.)*; Neo-Pergonal *(Fr.)*.

5931-v

Luteinising Hormone. Human Interstitial-cell-stimulating Hormone; Lutropin.

CAS — 9002-67-9; 39341-83-8 (human).

Units. 77 units of human pituitary luteinising hormone for immunoassay are contained in approximately 11.6 μg (with human albumin 1 mg and lactose 5 mg) in one ampoule of the first International Reference Preparation for Immunoassay (1974).

See also under Follicle-stimulating Hormone, above.

Preparations to replace the international reference preparation of human pituitary luteinising hormone for immunoassay are being considered. Highly purified alpha and beta subunits of human pituitary luteinising hormone are also being studied for their suitability as international reference materials.— Thirtieth Report of WHO Expert Committee on Biological Standardization, *Tech. Rep. Ser. Wld Hlth Org. No. 638*, 1979.

Luteinising hormone is extracted from the pituitary or urine of postmenopausal women. It is used together with follicle-stimulating hormone in the induction of follicular maturation in women being treated for failure of ovulation. Chorionic gonadotrophin (p.1268) has the actions of luteinising hormone and is the form in which it is generally used for other purposes.

5932-g

Serum Gonadotrophin *(B.P., B.P. Vet.).* Gonadotr. Seric.; PMSG; Pregnant Mares' Serum Gonadotrophin.

Pharmacopoeias. In *Aust., Br., Jap.*, and *Turk. Jap.* specifies not less than 1000 units per mg.

A dry sterile preparation of the glycoprotein fraction obtained from the plasma of mares in their sixtieth to seventy-fifth day of pregnancy, that stimulates the formation of follicles in the mammalian ovary.

A white or pale grey amorphous powder containing not less than 900 units per mg, calculated with reference to the dried substance, and losing not more than 10% of its weight on drying. Soluble in water. Store in a cool place in containers sealed to exclude micro-organisms and protected from light; under these conditions it is expected to retain its potency for not less than [illegible] years.

Units. 1600 units of equine serum gonadotrophin for bioassay, obtained from the serum of pregnant mares, is contained in approximately [illegible] (with lactose 5 mg) in one ampoule of the International Standard Preparation (1966).

Uses. Serum gonadotrophin has actions similar to those of follicle-stimulating hormone (p.1269); its clinical value is doubtful. Doses have ranged from 500 units intramuscularly weekly, to [illegible] units daily.

Preparations

Serum Gonadotrophin Injection *(B.P.).* A sterile solution of serum gonadotrophin prepared by dissolving, immediately before use, the contents of a sealed container (Serum Gonadotrophin for Injection) in the requisite amount of Water for Injections. The sealed container may contain added inert substances and bacteriostatic agents. pH of a solution containing 5000 units per ml [illegible] to 8.

Jap. P. includes a similar preparation.

Gonadotrophon FSH *(Paines & Byrne, UK).* Serum gonadotrophin, available as dry powder in ampoules containing 1000 units, with solvent.

Other Proprietary Names

Anteron *(Ger., S.Afr.)*; Elecgol *(Arg.)*; Gestyl *(Arg.)*; *Switz.)*; Primanuron *(Austral.)*; Seragon *(Ger.)*; Sc[illegible] *(Switz.)*.

Serum gonadotrophin was also formerly marketed in Great Britain under the proprietary name Gonadyl *(Organon)*.

5933-q

Growth Hormone. CB-311; HGH; STH; Somatotrophin; Somatropin. H-Phe-Pro-Thr-Ile-Pro-Leu-Ser-Arg-Leu-Phe-Asp-Asn-Ala-Met-Leu-Arg-Ala-His-Arg-Leu-His-Gln-Leu-Ala-Phe-Asp-Thr-Tyr-Gln-Glu-Phe-Glu-Glu-Ala-Tyr-Ile-Pro-Lys-Glu-Gln-Lys-Tyr-Ser-Phe-Leu-Gln-Asn-Pro-Gln-Thr-Ser-Leu-Cys-Phe-Ser-Glu-Ser-Ile-Pro-Thr-Pro-Ser-Asn-Arg-Glu-Glu-Thr-Gln-Gln-Lys-Ser-Asn-Leu-Gln-Leu-Leu-Arg-Ile-Ser-Leu-Leu-Leu-Ile-Gln-Ser-Trp-Leu-Glu-Pro-Val-Gln-Phe-Leu-Arg-Ser-Val-Phe-Ala-Asn-Ser-Leu-Val-Tyr-Gly-Ala-Ser-Asn-Ser-Asp-Val-Tyr-Asp-Leu-Leu-Lys-Asp-Leu-Glu-Glu-Gly-Ile-Gln-Thr-Leu-Met-Gly-Arg-Leu-Glu-Asp-Gly-Ser-Pro-Arg-Thr-Gly-Gln-Ile-Phe-Lys-Gln-Thr-Tyr-Ser-Lys-Phe-Asp-Thr-Asn-Ser-His-Asn-Asp-Asp-Ala-Leu-Leu-Lys-Asn-Tyr-Gly-Leu-Leu-Tyr-Cys-Phe-Arg-Lys-Asp-Met-Asp-Lys-Val-Glu-Thr-Phe-Leu-Arg-Ile-Val-Gln-Cys-Arg-Ser-Val-Glu-Gly-Ser-Cys-Gly-Phe-OH.

$C_{990}H_{1529}N_{263}O_{299}S_7$ = 22 124.

CAS — 9002-72-6; 12629-01-5 (human); 37267-05-3 (sheep).

A water-soluble protein from the anterior pituitary with an iso-electric point at pH 4.9. Human growth hormone consists of a single polypeptide chain of 191 amino acids. It is destroyed by boiling and by proteolytic enzymes.

The synthesis of human growth hormone fragments with growth-promoting activity.— F. Chillemi *et al.*, *Nature New Biol.*, 1972, *238*, 243.

Two immunoreactive components were identified in plasma or pituitary extracts of human growth hormone and named 'big' HGH and 'little' HGH. Little HGH was eluted as a globular protein of molecular weight 22 000 from Sephadex gel following filtration. Big HGH was less retarded by the gel and was considered to be twice the molecular weight. Big HGH made up about 37% of the total circulating material in healthy subjects and 8 to 14% in acromegalic patients. Big HGH showed much less activity in a radioreceptor assay than in a radioimmunoassay in both subjects and patients where there was no difference with little HGH.— P. Gerber *et al.*, *Science*, 1973, *182*, 829.

...synthesis of human growth hormone in bacteria by using synthetic DNA fragments and human pituitary material.— *J. Am. med. Ass.*, 1979, *242*, 701.

...synthetic sequence of human growth hormone.— *Can. med. Ass. J.*, 1979, *120*, 575.

...0.35 unit of human growth hormone for ...immunoassay is contained in approximately ...mg (with sucrose 5 mg and buffer salts) in ...ampoule of the first International Reference ...preparation for Immunoassay (1968).

Adverse Effects and Precautions. Antibodies have been formed in some patients. Because of the diabetogenic effect of growth hormone it should not be given to patients with diabetes mellitus.

Hypothyroidism has been reported during treatment with growth hormone.

Absorption and Fate. After intravenous injection growth hormone has a half-life of about 30 minutes.

References: M. L. Parker *et al.*, *J. clin. Invest.*, 1962, *41*, 263; B. J. Boucher (letter). *Nature*, 1966, *210*, 121.

Pregnancy and the neonate. Human growth hormone administered intravenously to 14 healthy pregnant women 270 and 10 minutes before delivery was not transferred across the placenta to the foetus.— Z. Laron *et al.*, *Acta endocr., Copenh.*, 1966, *53*, 687, per *J. Am. med. Ass.*, 1967, *199* (Feb. 13), A206.

Uses. Growth hormone is secreted by the anterior lobe of the pituitary. It promotes skeletal, visceral, and general body growth, stimulates protein anabolism, and affects fat and mineral metabolism. The hormone has a diabetogenic action on carbohydrate metabolism.

Growth hormone secretion is dependent on neural and hormonal influences including a hypothalamic release-inhibiting hormone (Somatostatin, GIF(77), a hypothalamic releasing factor (GHRF), not yet identified, and monoamines.

Somatomedins, formerly called sulphation factors, are polypeptides thought to mediate the effects of growth hormone on skeletal growth; they are formed in the liver and possibly the kidney.

Growth hormone is species specific, only that of human origin being effective in man.

In man, deficient secretion of growth hormone during the years of active body growth results in pituitary dwarfism. Overproduction of growth hormone before growth is complete results in gigantism. If it occurs after cessation of growth when the epiphyses have closed, it causes acromegaly, in which the features become coarse and the hands and feet become enlarged.

Growth hormone is given by injection in the treatment of pituitary dwarfism following assessment of pituitary function. Doses of 0.5 unit per kg body-weight have been given weekly by intramuscular injection in 2 or 3 divided doses.

Reviews of the actions and uses of growth hormone: *Lancet*, 1967, *1*, 257; J. A. Strong, *Practitioner*, 1968, *201*, 502; K. J. Catt, *Lancet*, 1970, *1*, 933; *Br. med. J.*, 1971, *2*, 236; *(ibid.*, 1971, *3*, 547; J. M. Tanner, *Nature*, 1972, *237*, 433; *Med. J. Aust.*, 1972, *2*, 457; *Med. Lett.*, 1973, *19*, 57; R. Shields, *Nature*, 1977, *267*, 308; P. C. Skowronska, *Postgrad. med. J.*, 1978, *54*, *Suppl.* 1, 91; R. G. G. Milner, *Archs Dis. Childh.*, 1979, *54*, 733; D. W. Cooke *et al.*, *Ann. Intern. Med.*, 1980, *92*, 650; *Br. med. J.* 1980, *280*, 270.

A review of somatomedins.— M. C. Stuart and L. Lazarus, *Med. J. Aust.*, 1975, *1*, 816. See also M. M. Daughaday and S. P. Nissley, *Nature*, 1977, *270*, 665.

The measurement of serum concentrations of somatomedin-C by radioimmunoassay appeared to be a useful adjunct to existing methods for the diagnosis of acromegaly. In 57 fasting acromegalic patients the mean serum concentration of immunoreactive somatomedin-C was 6.8 units per ml, 10 times the mean value in 48 healthy subjects, and was found to correlate significantly with clinical indicators of disease activity.— D. R. Clemmons *et al.*, *New Engl. J. Med.*, 1979, *301*, 1138. Somatomedin-C is probably identical to insulin-like growth factor I (IGF-I).— W. H. Daughaday, *ibid.*, 1175.

The intravenous infusion of pentagastrin resulted in a prompt and significant increase in plasma concentrations of growth hormone in 7 healthy male subjects; prolactin concentrations were not affected. This growth hormone-releasing property suggests a potential role for gastrin tetrapeptide as a local neurotransmitter in the hypothalamic-pituitary system. The peptide may be related to the growth hormone-releasing factor that has not yet been identified.— W. Domschke *et al.* (letter), *New Engl. J. Med.*, 1980, *303*, 458.

Effects on metabolism. A review of the mechanism of the diabetogenic effects of growth hormone.— J. Bornstein *et al.*, *Postgrad. med J.*, 1973, *49* (Mar.), *Suppl.*

The intravenous infusion of 4 mg of growth hormone over 30 minutes increased mean systemic concentrations of the hormone to 92.6 ng per ml. Plasma-glucose concentrations decreased by 9.5% and plasma concentrations of free fatty acids by 21.2% during the first hour. After 2 hours the free fatty acid concentrations rose to 145% of basal concentration. Intra-arterial perfusion did not produce early insulin-like effects nor stimulate lipolysis. Slight increases in growth-hormone concentrations had significant metabolic effects, including a 30% decrease in skeletal muscle sugar uptake and conversion of potassium output to uptake.— S. E. Fineberg and T. J. Merimee, *Diabetes*, 1974, *23*, 499, per *J. Am. med. Ass.*, 1974, *229*, 1368.

In a follow-up study of 26 dwarfs deficient in growth hormone, metabolic similarities to diabetic patients including grossly abnormal glucose tolerance and hyperlipidaemia were still apparent 10 years later. Unlike diabetics, retinopathy had not occurred and the prevalence of arteriosclerosis and hypertension was considerably lower in the dwarfs. Growth hormone may be one of the factors involved in clinical diabetic complications.— T. J. Merimee, *New Engl. J. Med.*, 1978, *298*, 1217. See also A. I. Winegrad and D. A. Greene, *ibid.*, 1250; R. W. Stout (letter), *ibid.*, *299*, 663.

Studies in 6 patients with isolated growth hormone deficiency demonstrated that insulin binding to monocytes was increased in such patients when compared with 8 controls. Insulin binding fell after treatment with growth hormone. These changes in binding were accompanied by similar changes in sensitivity to insulin.— V. Soman *et al.*, *New Engl. J. Med.*, 1978, *299*, 1025. The data on insulin binding in growth hormone deficiency are questionable and the study is being repeated.— P. Felig (letter), *ibid.*, 1980, *303*, 1120.

Further references: Z. Laron *et al.*, *Helv. paediat. Acta*, 1968, *23*, 37; M. Zachmann, *Schweiz. med. Wschr.*, 1969, *99*, 1125, per *J. Am. med. Ass.*, 1969, *209*, 1555; J. P. Alain *et al.*, *Metabolism*, 1977, *26*, 787.

Hand-Schüller-Christian disease. Five patients with Hand-Schüller-Christian disease were given growth hormone 2 units thrice weekly by intramuscular injection for 15 months to 2 years. All responded with a significant increase in growth-rate, which was greater in the 1st year than in the 2nd.— G. D. Braunstein *et al.*, *New Engl. J. Med.*, 1975, *292*, 332.

Obesity. A brief review of the use of growth hormone as therapy for obesity.— R. S. Rivlin, *New Engl. J. Med.*, 1975, *292*, 26.

Pituitary dwarfism. Fifteen children with growth-hormone deficiency were treated for at least 2 years with highly purified human growth hormone, 2 mg intramuscularly thrice weekly, increased to 4 mg thrice weekly if the growth-rate fell below 6 cm per year. Thyroid hormone was also given when necessary. During the first 6 months, the growth-rate increased twofold or more, but fell to or below the initial level when treatment was stopped. During the 1st and 2nd years, the average increases in height were 9 and 8.4 cm respectively. Seven children were given a third year of therapy and the average increase was 7 cm; increases of 7.8 and 7.2 cm occurred during the 4th and 5th years respectively in 1 child whose treatment was continued.— L. F. Soyka *et al.*, *J. clin. Endocr. Metab.*, 1970, *30*, 1.

In 12 children with hypopituitary dwarfism, growth occurred at a faster rate in 10 when they received growth hormone 0.1 unit per kg body-weight thrice weekly together with fluoxymesterone 2.5 mg per m^2 daily than when they received growth hormone alone.— M. H. MacGillivray *et al.*, *J. Am. med. Ass.*, 1972, *221*, 551.

A report of the first 5 years of a national study of the administration of growth hormone for 6 months in each year to patients with growth-hormone deficiency. The deficiency was idiopathic in 76% of the 151 patients and secondary to organic disease in 17%. The response was greater in younger patients and was dose-related; it was less in the later courses than during the 1st year but diminution was not progressive. Growth velocity increased in 9 of 11 patients with a decreased growth response when given fluoxymesterone.— H. Guyda *et al.*, *Can. med. Ass. J.*, 1975, *112*, 1301.

In a study of 17 children with growth abnormalities ..., hormone was given and withheld for alternating 6-month periods; some patients also received testosterone enanthate monthly. It was concluded that such children should receive growth hormone continuously throughout puberty and ideally until growth ceases. J. M. Tanner *et al.*, *J. Pediat.*, 1976, *89*, 1000. See also A. Aynsley-Green *et al.*, *J. Pediat.*, 1976, *89*, 992

A positive response in 2 children to growth hormone given for 18 months with an increase in growth-rate. Both children had congenital rubella with growth-hormone deficiency as well as deafness and retinopathy. — M. A. Preece *et al.*, *Lancet*, 1977, *2*, 842.

The efficacy of a depot gel preparation of growth hormone was evaluated in 15 children, aged between 7 and 15 years, with growth hormone deficiency. Plasma concentrations were measured in 6 patients following a single intramuscular injection of growth hormone 2 units, either in aqueous solution or in 15% gelatin solution (growth hormone gel). The peak plasma concentration of growth hormone was lower but more prolonged following administration of growth hormone gel. Studies in 6 patients indicated that intramuscular injection of growth hormone gel 2 units twice weekly is as effective as an aqueous solution of growth hormone 2 units thrice weekly, although the response to growth hormone treatment from either preparation is less in the second 12 months of therapy. A further 9 patients who were given growth hormone 0.3 units per kg body-weight weekly also showed diminished response to growth hormone in the second year of treatment. Use of a depot gel may reduce the amount of hormone and the frequency of injection required but does not solve the problem of a waning response to the long-term administration of growth hormone.— B. Lippe *et al.*, *Archs Dis. Childh.*, 1979, *54*, 609.

A study refuting the suggestion by W. Hamilton (*Archs Dis. Childh.*, 1979, *54*, 971) that human growth hormone provided in the UK by the Medical Research Council causes disproportionate osseous maturation for height gained, with ultimate stunting.— R. D. G. Milner *et al.*, *Archs Dis. Childh.*, 1980, *55*, 461.

Further references: P. H. Henneman, *J. Am. med. Ass.*, 1968, *205*, 828; A. Pertzelan *et al.*, *Clin. Endocr.*, 1976, *5*, 15; A. G. Kenion *et al.*, *J. Pediat.*, 1978, *92*, 491; D. Rudman *et al.*, *New Engl. J. Med.*, 1981, *303*, 123; M. A. Preece, *Br. med. J.*, 1981, *283*, 1145.

Turner's syndrome. A 14-year-old girl with Turner's syndrome and with normal concentrations of growth hormone grew 0.23 cm per month during about a year of observation. In the following year, during which she received growth hormone 10 mg weekly, the rate of growth was 0.5 cm per month; and in the third year, during which she received conjugated oestrogens 625 µg daily, the rate of growth was 0.3 cm a month.— M. Tzagournis, *J. Am. med. Ass.*, 1969, *210*, 2373.

A 9-year-old girl with Turner's syndrome and growth hormone deficiency grew only 2.3 cm during a year of observation. The next year she received growth hormone 5 units thrice weekly and grew 6.4 cm with bone age advancing proportionately.— C. G. D. Brook (letter), *New Engl. J. Med.*, 1978, *298*, 1203.

Proprietary Preparations

Crescormon *(KabiVitrum, UK).* Human growth hormone, available as sterile powder in vials of 4 units (about 2 mg), with 2-ml ampoules of sodium chloride injection. Store at 5°. (Also available as Crescormon in *Denm., Norw., S.Afr., Swed., Switz., USA*).

Nanormon *(Nordisk-UK, UK).* Human growth hormone, available as sterile powder in vials of 4 units (about 1.5 mg), with 2-ml ampoules of Water for Injection. Store at 2° to 8°. (Also available as Nanormon in *Denm., Norw.*).

Other Proprietary Names

Asellacrin *(USA)*; Crorm *(Ger., Ital.)*; Somacton *(porcine) (Ger.)*; Somatotrope Choay *(bovine) (Fr.)*.

5934-p

Lypressin. LVP. [8-L-Lysine]-vasopressin.
$C_{46}H_{65}N_{13}O_{12}S_2$=1056.2.
CAS — 50-57-7.

A stable, water-soluble, polypeptide which is usually prepared synthetically, or may be extracted from the posterior pituitary of pigs.

Units. 7.7 units of lysine-vasopressin are contained in approximately 23.4 µg of synthetic peptide (with albumin 5 mg and citric acid) in one

Bouveret, P., Sell, S. M., Gros, F. 1979. *Proc. Natl. Acad. Sci. USA* 76:5197–201
198. Sweeney, L. J., Clark, W. A., Umeda, P. K., Zak, R., Marnasck, F. J. 1984. *Proc. Natl. Acad. Sci. USA* 81:797–800
198a. Whalen, R. G., Sell, S. M., Eriksson, A., Thornell, L. E. 1982. *Dev. Biol.* 91:478–84
199. Obinata, T., Masaki, T., Takano-Ohmuro, H., Tanaka, T., Shimizu, N. 1983. *J. Biochem.* 94:1025–28
199a. Everett, A. W., Sinha, A. M., Umeda, P. K., Jakovcic, S., Rabinowitz, M., Zak, R. 1984. *Biochemistry* 23:1595–99
200. Lompré, A.-M., Nadal-Ginard, B., Mahdavi, V. 1984. *J. Biol. Chem.* 259:6437–46
201. Shani, M. 1985. *Nature* 314:283–86
202. Daubas, P., Robert, B., Garner, I., Buckingham, M. 1985. *Nucleic Acids Res.* 13:4623–43
203. Frank, G., Weeds, A. G. 1974. *Eur. J. Biochem.* 44:317–34
204. Simmen, R. C. M., Tanaka, T., Ts'ui, K. F., Putkey, J. A., Scott, M. J., et al. 1985. *J. Biol. Chem.* 260:907–12
205. Gilbert, W. 1985. *Science* 228:823–24
206. Umeda, P. K., Levin, J. E., Sinha, A. M., Cribbs, L. L., Darling, D. S., et al. 1986. See Ref. 164, pp. 809–23
206a. Gulick, J., Kropp, K., Robbins, J. 1985. *J. Biol. Chem.* 260:14513–20
207. Zezza, D. J., Heywood, S. M. 1986. *J. Biol. Chem.* 261:7455–60
208. Dibb, N. J., Brown, D. M., Karn, J., Moerman, D. G., Bolten, S. L., Waterston, R. H. 1985. *J. Mol. Biol.* 183:543–51
209. Eide, D. J., Anderson, P. 1985. *Mol. Cell. Biol.* 5:1–6
210. Eide, D., Anderson, P. 1985. *Genetics* 109:67–79
211. Anderson, P., Brenner, S. 1984. *Proc. Natl. Acad. Sci. USA* 81:4470–74
212. Eide, D., Anderson, P. 1985. *Proc. Natl. Acad. Sci. USA* 82:1756–60
213. Moerman, D. G., Benian, G. M., Waterston, R. H. 1986. *Proc. Natl. Acad. Sci. USA* 83:2579–83
214. Mogami, K., Fujita, S. C., Hotta, Y. 1982. *J. Biochem.* 91:643–50
215. Waterston, R. H., Smith, K. C., Moerman, D. G. 1982. *J. Mol. Biol.* 158:1–15
216. Waterston, R. H., Hirsh, D., Lane, T. R. 1984. *J. Mol. Biol.* 180:473–96
217. Landel, C. P., Krause, M., Waterston, R. H., Hirsh, D. 1984. *J. Mol. Biol.* 180:497–513
218. Miller, D. M., Maruyama, I. 1986. See Ref. 164, pp. 629–38
219. Asmundson, V. S., Julian, L. M. 1956. *J. Hered.* 47:248–52
220. Deleted in proof
221. Bulfield, G., Siller, W. G., Wright, P. A. L., Moue, K. J. 1984. *Proc. Natl. Acad. Sci. USA* 81:1189–92
222. Roderick, T. H., Davisson, M. T. 1984. See Ref. 169, pp. 343–55
222a. Davisson, M. T., Roderick, T. H. 1981. In *Genetic Variants and Strains of the Laboratory Mouse*, ed. M. C. Green, pp. 283–313. Stuttgart: Gustav Fischer Verlag
223. McKusick, V. A. 1986. *Clin. Genet.* 29:545–88
224. Deleted in proof
225. Engel, A. G. 1986. See Ref. 3, pp. 1185–240
226. Monaco, A. P., Neve, R. L., Colletti-Feener, C., Bertelson, C. J., Kurnit, D. M., Kunkel, L. M. 1986. *Nature* 323:646–50
227. Grimm, T. 1986. See Ref. 3, pp. 1214–50
228. Munsat, T. L. 1986. See Ref. 3, pp. 1251–66
229. Harper, P. S. 1986. See Ref. 3, pp. 1267–96
230. Wills, N., Gesteland, R. F., Karn, J., Barnett, L., Bolten, S., Waterston, R. H. 1983. *Cell.* 33:575–83
231. Karlik, C. C., Fyrberg, E. A. 1985 *Cell* 41:57–66
232. Moerman, D. G., Plurad, S., Waterston, R. H., Baillie, D. L. 1982. *Cell* 29:773–81

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INTERFERONS AND THEIR ACTIONS

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Natural Interferons

INTERFERON ALPHAS The purification of natural IFN-α was not only difficult for the reasons mentioned previously but was also complicated by the fact it was actually a family of structurally related molecules (19–23). The first purification of Hu-IFN-α's in the late 1970s made use of techniques such as immunoabsorbant affinity chromatography, SDS PAGE, and HPLC to achieve pure IFN preparations (19, 20, 24, 25). In general, the specific activities of the IFN-α's were 2–4 $\times$ 10^8 units per mg protein. Subsequently, multiple species of human interferon alpha have been purified by refinements of techniques discussed above from virus-induced buffy coats (19, 20, 24–29), Namalwa cells (55, 30–35), peripheral blood leukocytes from patients with chronic myelogeneous leukemia (20), and KG-1 cells (36). At present, the number of species of IFN-α isolated from each cell source ranges from 10 to greater than 16. In general, IFN-α's derived from different sources are quantitatively and/or qualitatively distinct from each other. The apparent molecular weights of the IFN-α's range from 16,000 to 27,000 (9, 19, 20, 24–36), and their amino acid compositions are very similar and rich in leucine and glutamic acid/glutamine (19, 20, 30, 31, 34, 36, 37). In addition, isoelectric focusing revealed the heterogeneous nature of IFN-α's; multiple species with pIs ranging from 5.5 to 6.5 have been observed (38). In general, natural human IFN-α's are polypeptides of 165–166 amino acids (19, 20, 30, 34, 36, 37), although two species have been isolated that lack the 10 COOH-terminal amino acid residues (39). Their amino acid sequences are quite homologous with at least 70% sequence identity between individual molecules (20, 22, 23, 26, 30–32, 37, 39–41). Particularly noteworthy is the conservation of the cysteines at positions 1, 29, 98/99, and 138/139. Based on studies with recombinant Hu-IFN-αA, these residues are involved in the formation of disulfide bonds (42–44). Three of the natural IFN-α's appear to be identical in sequence to two recombinant DNA–derived IFN-α's (21). Although some controversy existed concerning the presence of carbohydrate on IFN-α, it is now clear that several species of IFN-α are glycosylated; two species each from CML and KG-1 cells (45) and three from Namalwa cells (K. Zoon, unpublished data). The structure and linkage of the carbohydrate moiety are not known; however, it is postulated that the carbohydrate is linked to the protein through *O*-glycosylation, since all but one Hu-IFN-α sequences lack the sequence Asn-X-Ser/Thr necessary for N-glycosylation and the carbohydrate composition is consistent with O-glycosylation (45). A population of acid-labile Hu-IFN-α has been described and partially purified (46); the structure of this IFN is currently unknown.

The purification of Mu-IFN-α from virus-induced L929 cells (47, 48), C-243 cells (49), and mouse Ehrlich ascites cells (50, 51) was achieved in the late 1970s by the techniques discussed above, including ion exchange chromatography, gel filtration, controlled pore glass chromatography, immunoadsorbant affinity chromatography, affinity chromatography with ligands such as polynucleotides (Poly U) and octyl groups, isoelectric focusing, and SDS PAGE. The specific activities of the purified IFNs ranged from 4 $\times$ 10^8 to 3 $\times$ 10^9. It must be emphasized, however, that the specific activities are often not comparable from one laboratory to another because of different methods of protein determination. The apparent molecular weight of murine IFN-α is approximately 20,000. It appears to be a glycoprotein (51). As with the Hu-IFN-α's, the Mu-IFN-α's also belong to a family of structurally related proteins (52, 53). The amino acid composition of murine IFN-αC is very similar to that of the human and other murine IFN-α's (21, 53). The first 20 NH_2-terminal amino acid residues have been determined for murine IFN-αC (54). This portion of the primary sequence is similar to those derived from the cloned Mu-IFN-α's with 12 out of 20 residues being identical; it is also similar to that of Hu-IFN-α's (21). Monoclonal antibody affinity chromatography, gel filtration, and chromatofocusing have been used successfully to identify a minimum of five species of Mu-IFN-α's from L929 cells that have pIs ranging from 5.6 to 7.5 (55).

INTERFERON BETA Human IFN-β derived from diploid fibroblasts was the first IFN to be purified (56). Subsequently, improved procedures were developed to purify natural Hu-IFN-β, which included ion exchange chromatography, affinity chromatography (with lectins, phenyl groups, monoclonal antibodies against Hu-IFN-β and Cibacron Blue), and HPLC (57–63). Only one protein species was isolated and identified with these techniques; it exhibited an apparent molecular weight of approximately 20,000 and a specific activity of 2–5 $\times$ 10^8 units/mg protein. The amino acid composition is similar to that observed for Hu- and Mu-IFN-α's and Mu- and Bo-IFN-β (21, 41). The NH_2-terminal amino acid sequence for the first 19 residues of natural Hu-IFN-β was determined (59, 62–64) and is in agreement with the sequence derived from the DNA sequence (65). Natural Hu-IFN-β was demonstrated to be a glycoprotein by treatment of the molecule with a mixture of glycosidases (66) and the presence of amino sugars in purified IFN preparations (58, 62). It is believed that the major portion of the carbohydrate moiety is linked by *N*-glycosylation to the Asn at position 80. Based on the determination of target size for antiviral activity, the functional unit of Hu-IFN-β appears to be a dimer (67). Recently, another human IFN has been reported, which constitutes 5% of the IFN made by poly(I:C)-superinduced diploid fibroblasts, but appears to be structurally distinct from the major form of Hu-IFN-β (6, 68, 69). However, since this molecule shows no structural